

The Pollinator Research Task Force (PRTF) was formed in January 2016 and is comprised of nine pesticide registrants namely BASF Corp., Bayer Crop Science LP, Dow AgroSciences LLC DuPont Crop Protection, FMC Corp., Mitsui Chemicals Agro. Inc., Monsanto Co., Syngenta Crop Protection LLC and ValentUSA Corp. with the focus of mining and generating data to refine and improve pollinator risk assessments in North America and globally where applicable. One of the top priorities of the task force is to help coordinate a ring-test to validate the 22-d honey bee larval toxicity, repeated exposure method, as this study is currently being required to support new and existing uses of all pesticides in North America. This study has historically proven to be challenging to meet validity criteria and produce consistent results, therefore a proper validation of the method is necessary to be able to accurately interpret study data and appropriately inform risk assessments for immature honey bees.

The purpose of the ring test is to evaluate pupal survival through adult eclosion. The design is centered upon the current OECD Draft Guidance Document for the Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure” (OECD, 2015) with integrated method modifications developed at the University of Florida (UF). The UF modifications to the OECD methodology include changes to the diet composition (more water and less royal jelly in diets A and B to improve diet intake and limit drying out of the diet), the introduction of a pre-pupal transfer step (transferring of larvae on day 7/8 of development to a new culture plate), and changes to the rearing environment (no glycerol/sterilizing solution used, lid placed upon plate throughout development, and no emergence box).

Each participating laboratory will be asked to conduct the ring test during June or July 2016 and submit one test consisting of five increasing test concentrations of a toxic reference, a solvent control, and a water control. The toxic reference will be TGAI dimethoate (including a Certificate of Analysis) and will be provided by each participating laboratory. A minimum of 12 larvae from each of three colonies are allocated on the same plate for each treatment concentration and control. Participating laboratories are required to record biological observations (mortalities and other abnormal effects) on D4 to D8, D12, and at the time of adult eclosion (D17-D22). Analytical verification of the test diet will be required for the toxic reference stock and one concentration of test diet on D3 and D6. Collected samples will be sent to an independent laboratory for analysis. Temperature and relative humidity (RH) will be recorded during the entirety of the test and included as part of the complete data package. Tests will be conducted in June or July 2016 in order to standardize seasonal variability across all testing regions and laboratories.

A complete data package will be submitted to an independent consultant by the end of September 2016, including all biological observations, analytical reports, and raw temperature/RH data files. Data will be kept anonymous with all personally identifiable information from data sets removed prior to sending the data sets onto a committee. The committee will be comprised of four members representing industry, government, academia, and an independent consulting group that will be responsible for reviewing and summarizing the results of the ring-test

There will be no selection criteria to participate in the ring test in order to promote inclusion and transparency; however there will be quality standards that must be met by each participating laboratory to be included in the evaluation of pupal survival through adult eclosion. The quality standards will ensure that all participants submit all tests conducted as part of the ring test (regardless of the outcome) and are proficient at current larval toxicity guideline studies (eg. OECD 237). The following quality standards must be met for data to be included within ring-test performance evaluation.

- Larval survival on D7 for the average of the negative controls of the test must be $\geq 85\%$
- E-mail confirmation of the start date no later than the day after grafting
- All temperature/RH raw data from data logger will be submitted in final data package.

The PRTF will not provide any compensation to the ring-test participant; however the PRTF will work directly with an independent laboratory to cover fees for the analytical verification of the test diet samples. Analytical results will be provided to each ring-test participant for inclusion within their full data package.

Proposals should include the following specific information:

- Demonstration of experience to conduct the work
- Proposed timeline

Proposals responsive to this RFP should be received by the PRTF by no later than May 1, 2016. Electronic submissions are required. Proposals should be sent directly to Dan Schmehl (daniel.schmehl@bayer.com), co-lead for the PRTF larval toxicity, repeated exposure committee.